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(54) MATERIALS AND METHODS FOR BIOSYNTHESIS OF SERINE AND SERINE-RELATED PRODUCTS

MATERIALIEN UND VERFAHREN ZUR BIOSYNTHESE VON SERINEN UND SERINVERWANDTEN PRODUKTEN

MATIERES ET PROCEDES UTILISES POUR LA BIOSYNTHESE DE SERINE ET DE PRODUITS APPARENTES A LA SERINE

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(56) References cited:
EP-A- 0 190 921
EP-A- 0 401 735
EP-A- 0 418 840
US-A- 4 753 883

- **JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 264, no. 5, 15 February 1989, pages 2645-2648, Baltimore, US, D.J. SCHULLER et al.: "Enhanced expression of the Escherichia coli serA gene in plasmid vector. Purification, crystallization, and preliminary X-ray data of D-3 phosphoglycerate dehydrogenase", whole article**
- **JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 261, no. 26, 15 September 1986, pages 12179-12183, Baltimore, US, K.L. TOBEY et al.: "The nucleotide sequence of the serA gene of Escherichia coli and the amino acid sequence of the encoded protein, D-3-phosphoglycerate dehydrogenase", whole article (document cited in the description)**
- **BIOSCIENCE REPORTS, vol. 1, no. 9, 1981, pages 733-741, London, GB, G.A. GRANT et al.: "D-3-phosphoglycerate dehydrogenase from Escherichia coli: isolation by affinity chromatography and sequence comparison to other dehydrogenases", whole article**
- **JOURNAL OF BACTERIOLOGY, vol. 173, no. 5, March 1991, page 1571, Baltimore, US, K. O'Day et al.: "Physical locations of bglA and serA on the Escherichia coli K-12 chromosome", whole article**
- **JOURNAL OF MOLECULAR BIOLOGY, vol. 186, no. 4, 20 December 1985, pages 707-713, London, GB, R. CUNIN et al.: "Structure-function relationship in allosteric aspartate carbamoyltransferase from Escherichia coli. I. Primary structure of a pyrI gene encoding a modified regulatory subunit", whole article**

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Description

I. Background of the Invention

This invention relates to the general field of biosynthesis of serine and products related to serine, particularly tryptophan, and to methods and materials used in that biosynthesis.

Serine is a primary intermediate in the biosynthesis of a wide variety of cellular metabolites including such economically important compounds as choline, glycine, cysteine and tryptophan. In addition, serine acts as a single carbon donor and is responsible for 60 - 75% of the cell's total need for C_1 units through the production of 5,10-methylenetetrahydrofolate from tetrahydrofolate. These C_1 units are used in a wide variety of biosynthetic pathways including the synthesis of methionine, inosine monophosphate, other purines and some pyrimidines (e.g. thymidine and hydroxymethyl cytidine).

The serine biosynthetic pathway shown in Fig. 1 is generally available to a wide variety of tissues and microorganisms. The first committed step in that pathway is the conversion of 3-phospho-D-glyceric acid (PGA) to 3-phosphohydroxypyruvic acid (PHA) by means of the enzyme 3-phosphoglycerate dehydrogenase (PGD). The gene encoding PGD has been cloned and sequenced, and the amino acid sequence of the PGD subunit has been deduced. Tobey and Grant, J. Biol. Chem. 261:12179-12183 (1980).

In procaryotes (particularly bacteria) and microorganisms such as yeast, but not in higher eukaryotes, activity of wild-type PGD is inhibited by cellular serine levels. This inhibition has been studied kinetically and reportedly proceeds in an allosteric matter. Tobey and Grant, J. Biol. Chem., 261:12179-12183 (1986); Dubrow and Pizer, J. Biol. Chem. 252:1527-1551 (1977); McKitrick and Pizer, J. Bacteriol. 141:235-245 (1980).

Tosa and Pizer, J. Bacteriol. 106:972-982 (1971) studied the effect of a normally toxic serine analog, L-serine hydroxamate, on an E. coli strain. Selection on a growth medium containing that analog yielded serine resistant mutants. Some mutants were shown to have a modification in an enzyme unrelated to PGD, seryl-tRNA synthetase. Crude extract of one mutant showed PGD activity with reduced serine sensitivity (See J. Bacteriol. 106:972-982 (1971); Fig. 5; Table 6; and see p. 973 bottom left col., p. 977 bottom left col.).

II. Summary of the Invention

One aspect of the invention generally features DNA encoding 3-phosphoglycerate dehydrogenase (PGD) with reduced sensitivity to inhibition by serine in comparison to wild-type PGD--i.e., the DNA encodes PGD which has at least some level of enzymatic activity useful for biosynthesis, and which retains that activity at higher serine levels than does the (unmodified) wild-type PGD.

In preferred embodiments, the wild-type PGD is microbial or yeast PGD. The engineered DNA encodes PGD which comprises an alteration in the C-terminal 25% of wild-type PGD, preferably in the C-terminal 50 aminoacids. For example, the engineered DNA may encode PGD comprising a deletion in part or all of the C-terminus. Also preferably, the engineered DNA encodes PGD having an insertion in the C-terminus (e.g., between VAL363 and ASN364, or between ALA392 and GLN394) in addition to the deletion described above, or as a separate alteration.

The invention also features: a) expression vectors comprising the engineered DNA and regulatory DNA positioned and oriented for expression of the engineered DNA in a host expression system; b) cells comprising such expression vectors; and c) methods for producing serine or a serine-derived product by culturing such cells. As to b), above, the cell preferably is deleted for wild-type serA.

Yet another embodiment generally features a cell engineered (e.g., it includes a recombinant genetic construction) to produce a PGD-encoding mRNA transcript with an altered 3' end which transcript is translated by the cell to yield PGD with reduced sensitivity to inhibition by serine in comparison to wild-type PGD, said PGD comprising an alteration in the C-terminal 25% of wild-type PGD.

The invention provides decontrol of an important biosynthetic control point, thereby enhancing production of numerous compounds downstream of that point, including, in particular, serine and serine-derived products such as tryptophan. Other cellular metabolites derived from serine (i.e., serine is a primary intermediate in their biosynthesis) include choline, glycine, cysteine and C_1-donor-dependent compounds such as methionine, inosine monophosphate, purines, and some pyrimidines (e.g., thymidine and hydroxymethyl cytosine).

III. Description of the Preferred Embodiments

A. Drawings

Fig. 1 depicts steps in the biosynthesis of L-serine from glucose.

Fig. 2 is the sequence of the E. coli serA gene reported by Tobey and Grant (cited above) and the amino acid

sequence deduced from the gene.

Fig. 3 depicts bioconversion of L-serine and tetrahydrofolate to glycine and N^5,N^{10}-methylene tetrahydrofolate.

B. Providing Serine-Insensitive PGD

1. Genetically engineered constructions.

The preferred embodiments of the invention feature biosynthesis of serine and serine-related products--e.g., products described above derived by biosynthesis from serine. A first step in biosynthesis of these compounds according to the invention is the provision of serine insensitive PGD, as discussed below.

We have determined that there is a specific serine feedback mediating domain in PGD, and that domain can be altered to reduce serine sensitivity while maintaining useful levels of PGD activity. Fig. 2 shows one particular PGD genetic and amino acid sequence which can be used for reference in the following discussion. The sequence of Fig. 2 includes 410 amino acids (including the initial Met which is cleaved from the mature protein). The domain of PGD that can reduce serine sensitivity, without destroying PGD activity, is within the C-terminal 25% of the molecule, most preferably the 50 C-terminal residues.

Examples of PGD modifications are deletions of some or all of the C-terminal 42 amino acids, or insertions or substitutions within that region which reduce serine sensitivity while retaining useful PGD function. For example, insertion of amino acid residues between Val 363 and Asn 364 will increase the K_i of the PGD over wild-type, while retaining PGD activity.

More dramatic increases in K_i are accomplished by deleting some or all of the C-terminal amino acid residues. For example, deletion of the C-terminal residues GTIRARLLY and replacement with ASLD increases K_i by several orders of magnitude, while retaining a useful level of PGD activity. Other insertions are insertions between Ala392 and Gln394.

Other useful modifications include deletions from the C-terminus in addition to the insertions and modifications discussed above.

Genes encoding serine-insensitive PGD described above can be constructed by genetic engineering techniques that involve altering the 3' end of the coding region coding for the C-terminal amino acids, and then transforming a host strain with a vehicle to express the altered PGD enzyme.

Candidate altered enzymes are screened (as described below) for serine affinity (K_i) and for PGD activity by the methods generally discussed below.

2. Screening the genetically engineered constructions.

In screening genetic constructions made by the above-described methods, the following assays of PGD activity and of serine sensitivity are used.

While not critical to the invention, the assay of PGD activity is generally necessary in order to establish the degree of serine sensitivity of the altered enzyme. As is well known in the art, enzyme activity is a funtion of the total number of enzyme molecules and the catalytic activity of each molecule. Thus, in comparing the catalytic activity of PGD feedback variants, steps must be taken to adequately control for the relative number of PGD molecules for samples in which relative catalytic activity is to be compared. There are a number of ways in which this may be accomplished. However, since it is difficult to adequately establish the level of gene expression in cells transformed with truncated serA genes (due to decreased viability), the most suitable way to compare PGD activity produced from various constructs and the wild type is to chromosomally integrate the altered serA gene containing standard regulatory elements in a single copy, followed by harvesting the transformants and determination of the relative catalytic activity as compared to PGD from wild type cells.

Any method suitable for the measurement of PGD activity may be employed. PGD activity may be measured through detection of either the forward or the reverse reaction by the method of McKitrick, John C. and Lewis I. Pizer. (1980) J. Bacteriol. 141 235-245.

The enzymatic assay described above is suitable for determination of serine sensitivity for any PGD enzyme, including those with chemically modified C-termini. The assay is performed in the presence of various levels of serine. The catalytic activity in the presence of serine is compared to catalytic activity in the absence of serine, and the K_i calculated.

In most cases it will be preferred to reduce serine sensitivity without significantly altering PGD catalytic activity. In still other embodiments it may be desirable to reduce both the feedback sensitivity and the catalytic activity. The constructions having a C-terminal amino acid sequence of 3-phosphoglycerate dehydrogenases listed in Table 1 (described below) may be used.

Table 1

C-terminal Amino Acid Sequences of 3-phosphoglycerate dehydrogenases

SerA	Sequence	ki/μm	Units
WT	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- -IRA RLLY (SEQ. ID NO: 1)	<0.1	.05
1455	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PASL D (SEQ. ID NO: 2)	>100	<.01
1459	AEQG VLV (SEQ. ID NO: 3)	>100	N/A
1507	AEQG VL (SEQ. ID NO: 4)	>100	N/A
1508	AEQG VCSR ANIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- -IRA RLLY (SEQ. ID NO: 5)	3.8	.05
1509	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL SRQA MKAI PGT- -IRA RLLY (SEQ. ID NO: 6)	>100	N/A
1510	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL L	>100	N/A

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	(SEQ. ID NO: 7)		N/A
1511	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- AIRA RLLY	>100	
	(SEQ. ID NO: 8)		N/A
1512	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PVL	>100	
	(SEQ. ID NO: 9)		N/A
1530	AEQG V--- ---- ---- ---- ---------- ---- ---- -CSR AIRA RLLY	>100	
	(SEQ. ID NO: 10)		N/A
1531	AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL ---- ---- --SR AIRA RLLY	>100	
	(SEQ. ID NO: 11)		

Other constructions with modifications in the 3' 25% of the coding sequence also fall within the scope of the present

invention since it is a simple matter to prepare test constructs and transform cells according to the present invention and test for serine inhibition of PGD activity.

Any vector which leads to expression of a PGD protein having an alteration in the C-terminal 25% of wild type PGD, lacking sensitivity to inhibition by serine pertains to the present invention. In general, however, in the absence of a sink for serine, high levels of expression of feedback free PGD should be avoided since the resulting high cytoplasmic levels of serine or serine-derived metabolites can be toxic to the cell. Thus, in general, for any construct coding for a feedback inhibited PGD with normal catalytic activity and expression levels similar to those from the native gene, transformation will likely lead to high levels of PGD expression and decreased cellular viability. The toxicity of high levels of serine produced may in fact select for mutants with decreased PGD expression. Thus while transformation using multi-copy plasmids may be useful in initial screening of constructs with some embodiments, it is preferred to chromosomally integrate serA constructs in single copies into the genome. Additionally, chromosomal integration as described below facilitates activity measurement of the feedback deleted PGD. Thus, in most embodiments where strong catalytic activity is expected or desired, it is preferred to utilize vectors suitable for single copy chromosomal integration. Many such vectors and strategies for their use are known in the art. Useful vectors and constructs can be made to allow for the successful transformation and expression of the enzyme in an appropriate host for producing the desired product. Means for accomplishing these ends are well known to those familiar with the art and are not central to the present invention. In addition to the altered PGD-encoding DNA, the expression vector will contain various other elements described below.

First, the coding sequences present on the vector will be accompanied by the appropriate regulatory elements necessary for the appropriate level of expression of the coding sequences, including promoters, ribosome binding sites, and termination sequences. In most cases, the native serA regulatory sequences will be the preferred source of the catalytically active part of the molecule, although it is recognized that many other regulatory sequences known to the art or yet to be discovered may be employed.

Second, it is preferred that sequences encoding selective markers and/or reporter genes, along with the appropriate regulatory elements, will also be present on the vector. The expression of such selective markers is useful in identifying transformants. Appropriate selective marker genes include those coding for ampicillin, tetracycline, and chloramphenicol.

Third, the desirability of an origin of replication on the plasmid vector depends largely on the desirability of maintaining the genes chromosomally or extrachromosomally. Those familiar with the art appreciate the various strategies by which the lack of an origin of replication can be exploited to promote integration into the chromosome. See, e.g., Backman et al., U.S. Patent 4,743,546.

Once the expression vector is constructed, a suitable host cell can be transformed with a vector containing a transcription unit coding for a serine insensitive PGD protein. In most cases, it is useful to employ cells for which the endogenous PGD protein is known to be inhibited by serine and in which the endogenous serA gene is deleted and replaced by the altered gene of the invention. Such cell systems are useful for the overproduction of serine-related metabolites. Cells known to contain serine sensitive proteins are prokaryotes and yeasts.

The following example illustrates, but does not limit, the invention.

Example 1

Construction of serA Gene Alleles Encoding Feedback Resistant 3-phosphoglycerate Dehydrogenases

The E. coli K12 serA gene was isolated on a 6.4 Kb DNA fragment from a sau3A partial digest cloned into the BclI site of pTR264. See, Roberts et al., Gene 12:123 (1980). This plasmid was named pKB1302. A 3 Kb SalI to SphI fragment of pKB1302 DNA containing the serA gene was cloned into pUC19 to generate pKB1321. pKB1370 was generated by cloning a 3 Kb HindIII to SalI fragment containing the serA gene into pBR322.

Alleles of serA encoding feedback resistant 3-phosphoglycerate dehydrogenases were generated by inserting XbaI linkers at resctriction sites in the 3' region of the serA gene. A partial digest of plasmid pKB1321 by HincII yielded blunt ends at position 1793, where insertion of linkers gave: a) pKB1459, encoding a truncated 3-phosphoglycerate dehydrogenase; b) pKB1507, encoding a truncated 3-phosphoglycerate dehydrogenase; and c) pKB1508 which encodes a 3-phosphoglycerate dehydrogenase with a four amino acid residue insert.

PstI digestion of pKB 1321 gives a 3' overhang at position 1888. Blunt ends were generated by the action of the Klenow fragment of DNA polymerase I. Linkers were ligated into the blunt end fragments and the derived plasmids were pKB1509 which encodes a 3-phosphoglycerate dehydrogenase with a two-amino-acid insert and pKB1510 which encodes a truncated 3-phosphoglycerate dehydrogenase. A KpnI digest of pKB1370 was made blunt ended with Klenow fragment of DNA polymerase I and inserted linkers yielded plasmids encoding truncated 3-phosphoglycerate dehydrogenase, pKB1455 and pKB1512, or 3-phosphoglycerate dehydrogenase with a two amino acid residue insert, pKB1511. Deletion plasmids pKB1530 and pKB1531 were generated by inserting the 0.8 Kb BamHI to XbaI fragment from pKB1508 or the 0.9 Kb BamHI to XbaI fragment from pKB1509 respectively, into the 5.8 Kb BamHI to XbaI fragment of pKB1511.

The following Table 2 summarizes the various constructs made.

Table 2

SerA Allele	Plasmid	Restriction Site	Linker	Result
Ser A 1455	pKB 1370	Kpn I	CTAGTCTAGACTAG (SEQ. ID NO: 12)	Truncated
Ser A 1459	pKB 1321	Hind II	CTAGTCTAGACTAG (SEQ. ID NO: 13)	Truncated
Ser A 1507	pKB 1321	Hind II	CTCTAGAG (SEQ. ID NO: 14)	Truncated
Ser A 1508	pKB 1321	Hind II	TGCTCTAGAGCA (SEQ. ID NO: 15)	Insert
Ser A 1509	pKB 1321	Pst I	GCTCTAGAGC (SEQ. ID NO: 16)	Inser
Ser A 1510	pKB 1321	Pst I	TGCTCTAGAGCA (SEQ. ID NO: 17)	Truncated

Ser A 1511	pKB 1370	Kpn I	GCTCTAGAGCA (SEQ. ID NO: 18)	Insert
Ser A 1512	pKB 1370	Kpn I	TGCTCTAGAGC (SEQ. ID NO. 19)	Truncated
Ser A 1530	pKB 1511 + pKB 1508	Hind II + Kpn I		Deleted
Ser A 1531	pKB 1511 + pKB 1509	Pst I + Kpn I		Deleted

For all the constructs, the starting vector, the restriction site used, and the sequence of the inserted linker are indicated. The Ki values for serine are given in Table 1 as well as the relative catalytic activity for three of these constructs following chromosomal integration (described below). N/A indicates that the construction was not chromosomally inte-

grated, and the activity level therefore was not standardized.

3. Chemical Modifications

Those skilled in the art will understand that deletions or modifications of the C-terminus of wild-type PGD can be accomplished enzymatically or chemically, e.g. by various carboxypeptidases including carboxypeptidase Y or by lactoperoxidase mediated iodination.

4. Use of Antisense mRNA

Alternatively, it may be possible to reduce serine sensitivity in vivo through the generation of PGD-encoding transcripts truncated at the 3' end by means of the producing antisense mRNAs that include nucleotide sequences complementary to portions of the 3' coding region of native or transformed PGD coding sequences.

C. Production of Desired Compounds

As shown in Fig. 3, serine is an intermediate in the production of glycine. It is also an intermediate in the production of N^5,N^{10}-methylenetetrahydrofolate which is generalized C_1 donor essential for synthesis of methionine, purines (including inosine) and some pyrimidines. Thus, the over-production of serine from phosphoglycerate may be useful in a wide range of bacterial production systems including production systems for choline, glycine, cysteine, methionine, tryptophan, and all purines including inosine monophosphate.

The following specific examples illustrate the invention.

Example 2

Host Strain Preparation

Sequences internal to a plasmid born serA gene were replaced with a kanamycin resistance gene. This plasmid was then used to inactivate the host strain serA gene by means of allele exchange as follows:

The serA region of YMC9 (ATCC33920) was cloned from chromosomal DNA, partially digested with Sau 3AI, by complementation of PCI523 (argI61, argF58, serA27, purA54, thr-25, tonA49, relA1, spoT1), obtained from Coli Genetic Stock Center, Yale University, New Haven, CT. A 3 kb fragment carrying the serA gene was subcloned into pUC19 giving rise to a plasmid called pKB1321. From this plasmid a 3kb SalI to HindIII fragment was recloned into pBR322 giving rise to plasmid pKB1370. The KpnI site at the 3' end of the serA gene was converted to BamHI with a linker and the BamHI fragment internal to the resulting serA was replaced with the BamHI fragment from pUC-4-KSAC (Pharmacia) containing the Tn903 kanamycin resistance gene. This new plasmid was designated pKB1429. A pBR322 derivative called pKB 701 (ATCC 39772), was generated in which the MboI and TThIII 1 flanking the origin of replication were converted to KpnI sites. The SalI to EcoRI fragment containing serA::KanR from pKB1429 was cloned into pKB701 giving rise to pKB1438. pKB1438 was digested with KpnI to remove the ori region. The large fragment containing the ampicillin resistance coding region as well as the serA::KanR was circularized and used in a $CaCl_2$ transformation of YMC9. Following transformation, the host YMC9 cells were placed under selection on ampicillin. Under these conditions, ampicillin resistant clones develop by incorporation of the circular DNA through homologous recombination in the serA gene flanking regions. Growth of the ampicillin resistant isolate in the absence of ampicillin selection results in loss of the ampicillin resistance gene by homologous recombination of the repeated sequences of serA gene flanking regions. Such strains were identified by the loss of production of β-lactamase using AmpScreen (BRL) according to the manufacturer's directions. Duplicate streaking of single colonies on media in the presence and absence of serine revealed ampicillin sensitive clones requiring serine for growth on minimal medium and which were also resistant to kanamycin. One such isolate was named KB875.

Example 3

Chromosomal Integration of Altered serA Sequences by Allele Exchange

The serA1455 allele was introduced to the chromosome by a process analogous to that used for the introduction of serA::KanR as outlined in example 2. Briefly, a fragment (SalI to HindIII) bearing the serA1455 allele was cloned into pkB701. The plasmid origin was removed by KpnI digestion. The circularized DNA was used to tranform to ampicillin resistance giving rise to a strain designated. After non-selective growth, using Ampscreen and replica plating for kanamycin, KB904 (serA1455) was isolated and shown to be sensitive to ampicillin and kanamycin KB904. The resulting

serA1544 allele can be transferred into production strains by P1 transduction. Miller (1972) Experiments In Mol. Genetics Cold Spring Harbor Press, pp. 201-205.

Example 4

Chromosomal Integration of Altered serA Sequences by recD Dependent Gene Replacement

Another approach was utilized to move the serA1508 allele on to the chromosome. The strain KB875 was made recD by P1 transduction from V220 (recD, argA:Tn10. Amundsen et al., (1986) Proc. Acad. Sci., U.S.A. 82 5558-5562) (DSM 6823). The gene for an essential third subunit of exonuclease V. to give JGP101. The plasmid pKB1508 was linearized and used to transform JGP101 to serine prototrophy essentially as described by Shevell et al., (1988) J. Bacteriol. 170 3294-3296, to give JGP103. The serA1508 allele can then be moved to production strains by P1 transduction. Miller et al., Experiments in Mol. Genetics Cold Spring Harbor Lab., pp. 201-205 (1972).

Harvesting of Overproduced Metabolites

For the overproduction of serine-related metabolites, cells can be prepared which produce PGD with reduced serine sensitivity, and grown in fermentors under the appropriate conditions, in most cases to stationary phase. The cells will then be harvested and lysed and the desired metabolite prepared according to standard biochemical procedures. Conditions, principles, and references for the growth of microbes, and the harvesting of specific metabolites are provided by Crueger and Crueger (1982) (Biotechnology: A Textbook of Industrial Microbiology) and Herrmann and Somerville (1983) (Amino Acids: Biosynthesis and Genetic Regulation).

Claims

1. Engineered DNA construct encoding 3-phosphoglycerate dehydrogenase (PGD) with reduced sensitivity to inhibition by serine in comparison to wild-type PGD, in which said construct comprises DNA encoding PGD, said PGD comprising an alteration in the C-terminal 25% of wild-type PGD.

2. The engineered DNA construct of claim 1 in which said PGD, comprises an alteration in the C-terminal 50 amino acids of wild-type PGD.

3. The engineered DNA construct of claim 1 in which said PGD comprises a C-terminal deletion of wild-type PGD.

4. The engineered DNA construct of claim 1 in which said C-terminal alteration comprises an insertion into a wild-type PGD sequence.

5. The engineered DNA of claim 4 wherein said insertion is between VAL 363 and ASN 364 or between ALA 392 and GLN 394 of wild-type PGD.

6. The engineered DNA construct of claim 1 in which said DNA encodes PGD having one of the following amino acid sequences

```
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PASL D;
AEQG VLV;
AEQG VL;
AEQG VCSR ANIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- -IRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL SRQA MKAI PGT- -IRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL L;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- AIRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PVL;
AEQG V--- ---- ---- ---- ---- ---- ---- ---- -CSR AIRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL ---------- --SR AIRA RLLY;
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instead of the 52 C-terminal amino acids of the wild type PGD.

7. An expression vector comprising the engineered DNA construct of any one of claims 1-6 and regulatory DNA positioned and oriented for expressing of said engineered DNA in a host expression system.

8. A cell comprising the engineered DNA of any one of claims 1-6 and regulatory DNA positioned and oriented to express said engineered DNA in said cell.

9. The cell of claim 8 in which said cell is deleted for wild-type serA.

10. A method for producing a desired product which is serine or serine-derived, comprising culturing a cell according

to claim 8 and recovering said desired product.

11. The method of claim 12 in which said product is serine.

Patentansprüche

1. Verändertes DNA-Konstrukt, das für 3-Phosphoglyceratdehydrogenase (PGD) kodiert, die über eine im Vergleich zur Wildtyp-PGD verringerte Empfindlichkeit gegen Hemmung durch Serin verfügt, wobei dieses Konstrukt eine DNA umfaßt, die für PGD mit einer Modifikation in den C-terminalen 25% der Wildtyp-PGD kodiert.

2. Verändertes DNA-Konstrukt nach Anspruch 1, wobei diese PGD eine Modifikation in den C-terminalen 50 Aminosäuren der Wildtyp-PGD umfaßt.

3. Verändertes DNA-Konstrukt nach Anspruch 1, wobei diese PGD eine C-terminale Deletion der Wildtyp-PGD umfaßt.

4. Verändertes DNA-Konstrukt nach Anspruch 1, wobei diese C-terminale Modifikation eine Insertion in eine Wildtyp-PGD-Sequenz umfaßt.

5. Veränderte DNA nach Anspruch 4, dadurch gekennzeichnet, daß diese Insertion zwischen VAL 363 und ASN 364 oder zwischen ALA 392 und GLN 394 der Wildtyp-PGD vorgenommen wird.

6. Verändertes DNA-Konstrukt nach Anspruch 1, wobei diese DNA für PGD mit einer der folgenden Aminosäuresequenzen

```
AEQG V---  -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PASL D;
AEQG VLV;
AEQG VL;
AEQG VCSR ANIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- -IRA RLLY;
AEQG V---  -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL SRQA MKAI PGT- -IRA RLLY;
AEQG V---  -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL L;
AEQG V---  -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PGT- AIRA RLLY;
AEQG V---  -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --QA MKAI PVL;
AEQG V---  ----  ----  ----  ----  ----  ---------  ---------  -CSR AIRA RLLY;
AEQG V---  -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL ---------  --SR AIRA RLLY;
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statt der 52 C-terminalen Aminosäuren der Wildtyp-PGD kodiert.

7. Expressionsvektor mit dem veränderten DNA-Konstrukt nach einem der Ansprüche 1-6 und Regulator-DNA in einer Position und Orientierung für die Expression dieser veränderten DNA in einem Wirtsexpressionssystem.

8. Zelle mit der veränderten DNA nach einem der Ansprüche 1-6 und Regulator-DNA in einer Position und Orientierung, um diese veränderte DNA in dieser Zelle zu exprimieren.

9. Zelle nach Anspruch 8, wobei diese Zelle eine Deletion für Wildtyp-serA trägt.

10. Verfahren zur Herstellung eines gewünschten Produkts, bei dem es sich um Serin oder um ein Serinderivat handelt,

dadurch gekennzeichnet, daß man eine Zelle nach Anspruch 8 zūchtet und dieses erwūnschte Produkt gewinnt.

11. Verfahren nach Anspruch 12, wobei es sich bei dem Produkt um Serin handelt.

Revendications

1. Construction élaborée d'ADN, codant la 3-phosphoglycérate déshydrogénase (PGD) avec une sensibilité réduite à l'inhibition par la sérine par rapport à la PGD de type sauvage, dans laquelle la construction comprend l'ADN codant la PGD, la PGD comprenant une altération dans les 25% C-terminaux de la PGD de type sauvage.

2. Construction élaborée d'ADN suivant la revendication 1, dans laquelle la PGD comprend une altération dans les 50 acides aminés C-terminaux de la PGD de type sauvage.

3. Construction élaborée d'ADN suivant la revendication 1, dans laquelle la PGD comprend une délétion C-terminale de la PGD de type sauvage.

4. Construction élaborée d'ADN suivant la revendication 1, dans laquelle l'altération C-terminale comprend une insertion dans une séquence de PGD de type sauvage.

5. Construction élaborée d'ADN suivant la revendication 4, dans laquelle l'insertion se situe entre VAL 363 et ASN 364 ou entre ALA 392 et GLN 394 de la PGD de type sauvage.

6. Construction élaborée d'ADN suivant la revendication 1, dans laquelle l'ADN code une PGD ayant l'une des séquences en acides aminés suivantes :

```
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --
QA MKAI PASL D;
AEQG VLV;
AEQG VL;
AEQG VCSR ANIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --
QA MKAI PGT- -IRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL
SRQA MKAI PGT- -IRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL L;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --
QA MKAI PGT- AIRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL --
QA MKAI PVL;
AEQG V--- ---- ---- ---- ---- ---- ---- -------- ---
----- -CSR AIRA RLLY;
AEQG V--- -NIA AQYL QTSA QMGY VVID IEAD EDVAEKAL ---
----- --SR AIRA RLLY;
```

à la place des 52 acides aminés C-terminaux de la PGD de type sauvage.

7. Vecteur d'expression comprenant la construction élaborée d'ADN, suivant l'une quelconque des revendications 1 à 6, et un ADN de régulation placé et orienté pour l'expression de l'ADN élaboré dans le système d'expression d'un hôte.

8. Cellule contenant l'ADN élaboré suivant l'une quelconque des revendications 1 à 6, et un ADN de régulation placé

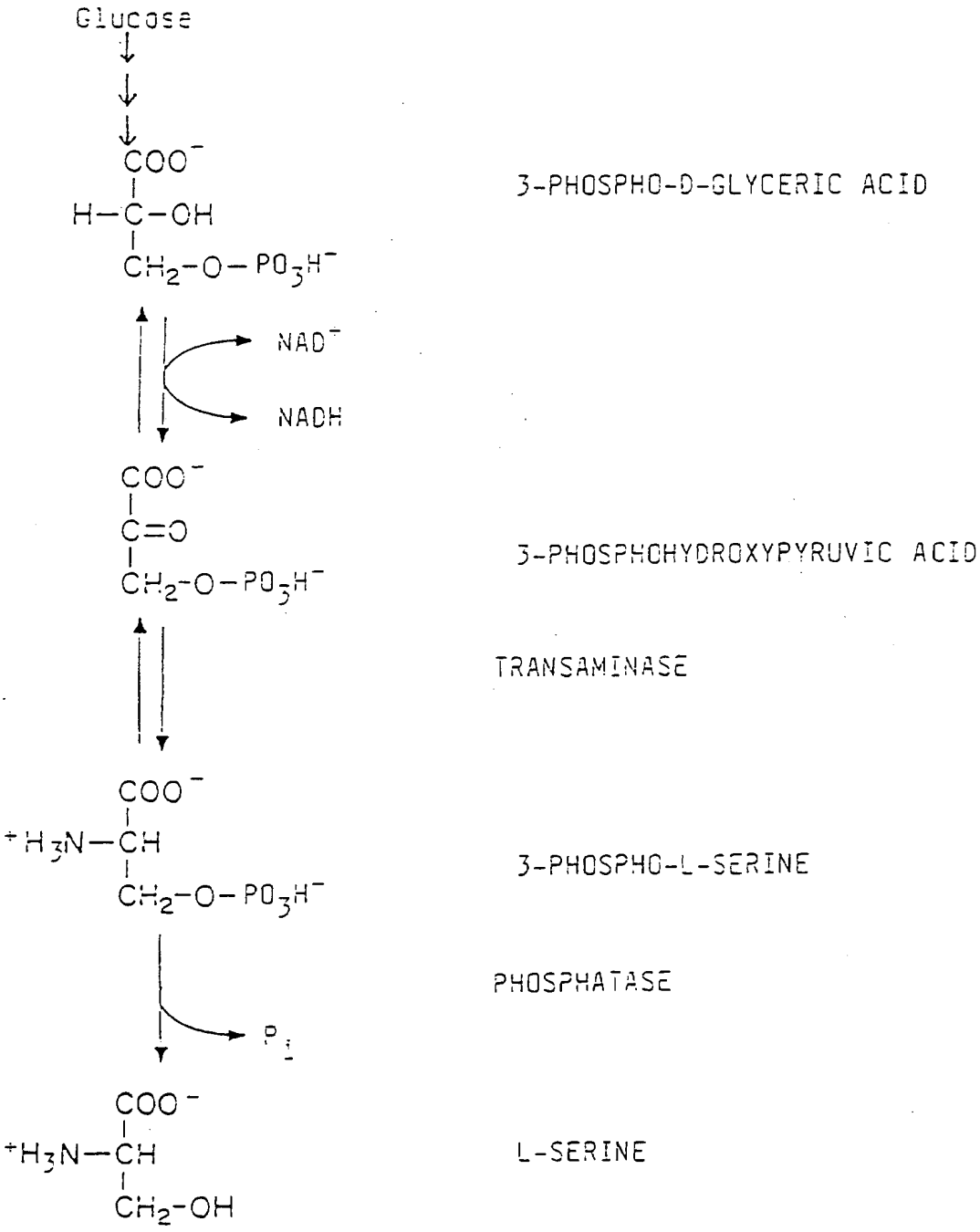
et orienté pour exprimer l'ADN élaboré dans la cellule.

9. Cellule suivant la revendication 8, dans laquelle la cellule ne comprend pas de serA de type sauvage.

10. Procédé de production d'un produit souhaité qui est la sérine ou un dérivé de la sérine, comprenant la mise en culture d'une cellule suivant la revendication 8 et la récolte du produit souhaité.

11. Procédé suivant la revendication 12, dans lequel le produit est la sérine.

Fig. 1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CH₂—O—PO₃H⁻
NAD⁻
NADH
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COO⁻
C=O
CH₂—O—PO₃H⁻
3-PHOSPHOHYDROXYPYRUVIC ACID
TRANSAMINASE
COO⁻
⁺H₃N—CH
CH₂—O—PO₃H⁻
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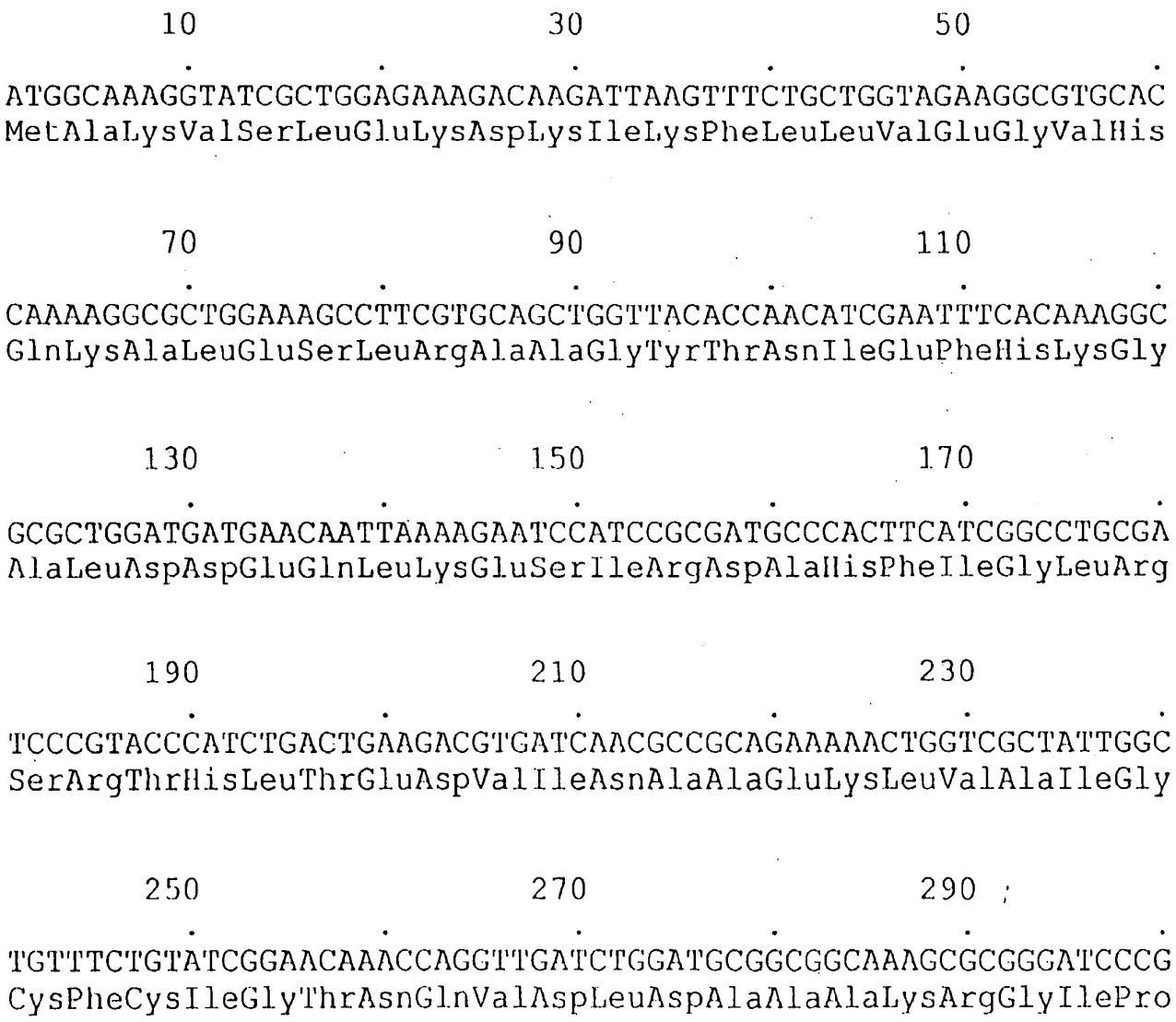
PHOSPHATASE
Pi
COO⁻
⁺H₃N—CH
CH₂—OH
L-SERINE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90
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150
170
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190
210
230
TCCCGTACCCATCTGACTGAAGACGTGATCAACGCCGCAGAAAAACTGGTCGCTATTGGC
SerArgThrHisLeuThrGluAspValIleAsnAlaAlaGluLysLeuValAlaIleGly
250
270
290
TGTTTCTGTATCGGAACAAACCAGGTTGATCTGGATGCGGCGGCAAAGCGCGGGATCCCG
CysPheCysIleGlyThrAsnGlnValAspLeuAspAlaAlaAlaLysArgGlyIlePro

Fig. 2 - 1

```
        310                 330                 350
         .                   .                   .                   .
GTATTTAACGCACCGTTCTCAAATACGCGCTCTGTTGCGGAGCTGGTGATTGGCGAACTG
ValPheAsnAlaProPheSerAsnThrArgSerValAlaGluLeuValIleGlyGluLeu

        370                 390                 410
         .                   .                   .                   .
CTGCTGCTATTGCGCGGCGTGCCGGAAGCCAATGCTAAAGCGCACCGTGGCGTGTGGAAC
LeuLeuLeuLeuArgGlyValProGluAlaAsnAlaLysAlaHisArgGlyValTrpAsn

        430                 450                 470
         .                   .                   .                   .
AAACTGGCGGCGGGTTCTTTTGAAGCGCGCGGCAAAAAGCTGGGTATCATCGGCTACGGT
LysLeuAlaAlaGlySerPheGluAlaArgGlyLysLysLeuGlyIleIleGlyTyrGly

        490                 510                 530
         .                   .                   .                   .
CATATTGGTACGCAATTGGGCATTCTGGCTGAATCGCTGGGAATGTATGTTTACTTTTAT
HisIleGlyThrGlnLeuGlyIleLeuAlaGluSerLeuGlyMetTyrValTyrPheTyr

        550                 570                 590
         .                   .                   .                   .
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```
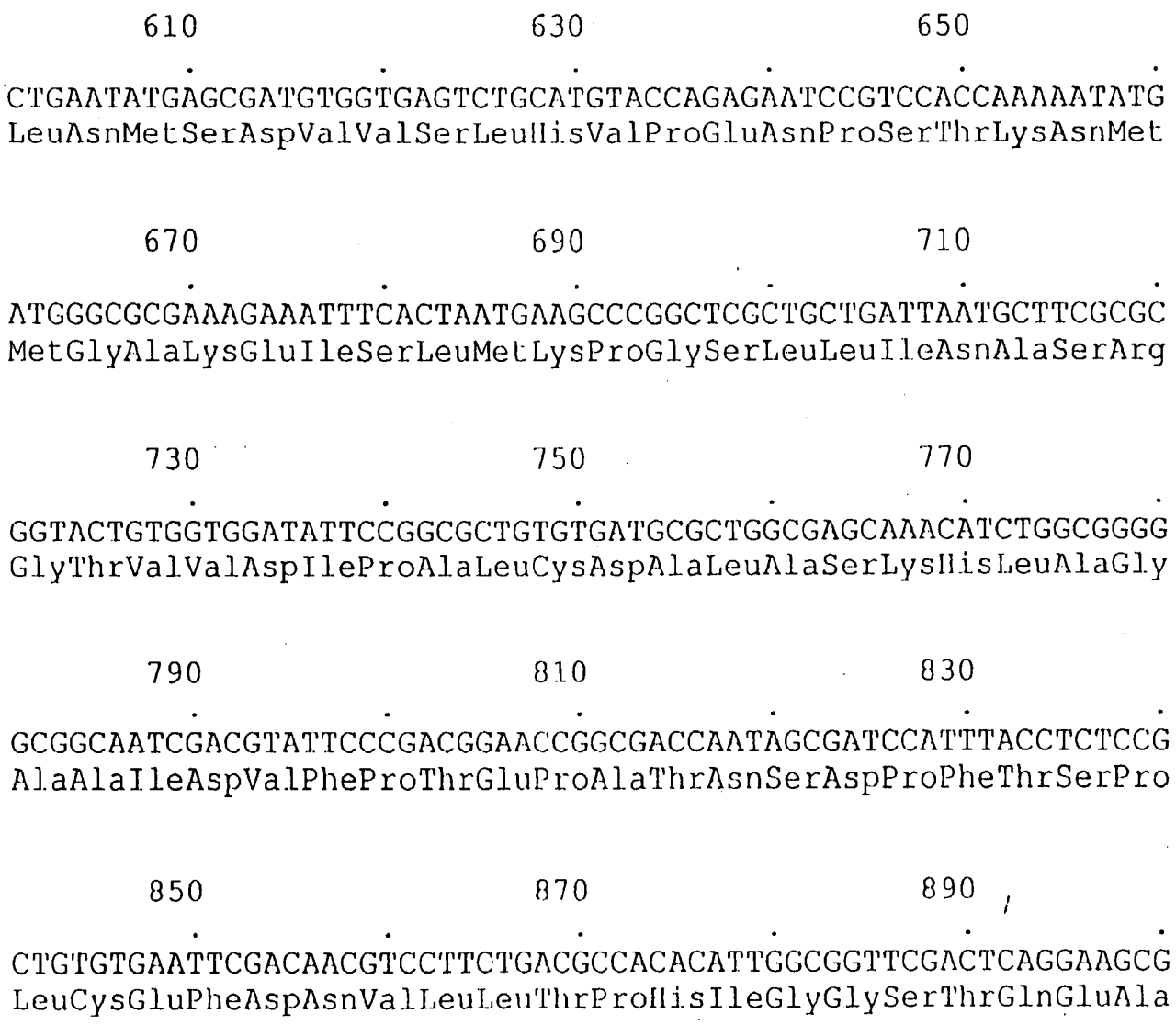

Fig. 2 - 2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690
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750
770
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790
810
830
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850
870
890
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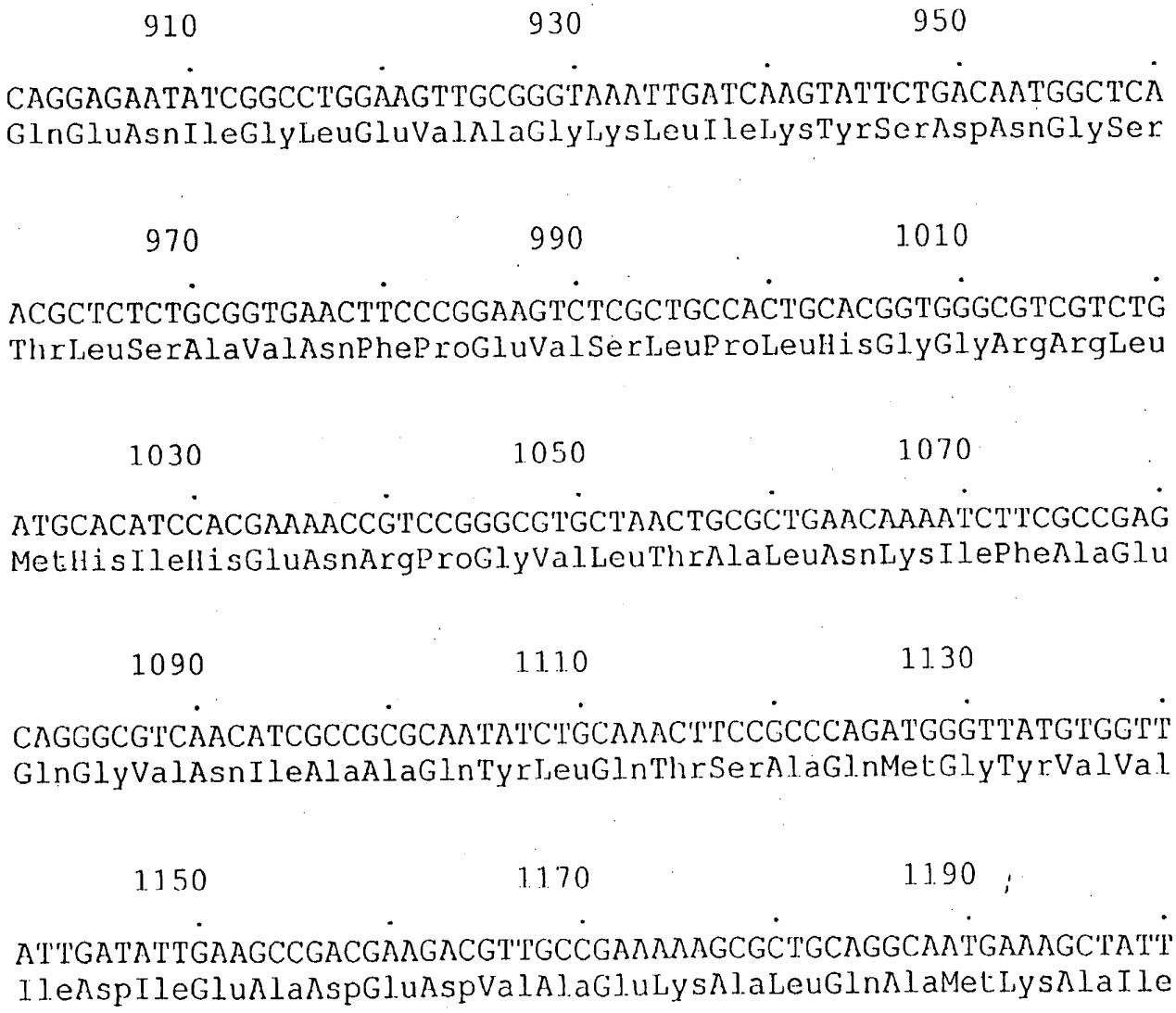
Fig. 2 - 3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1010
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1070
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1090
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1130
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1170
1190
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Fig. 2 - 4

Fig. 2 - 5

```
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                    .
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ProGlyThrIleArgAlaArgLeuLeuTyrEnd
```
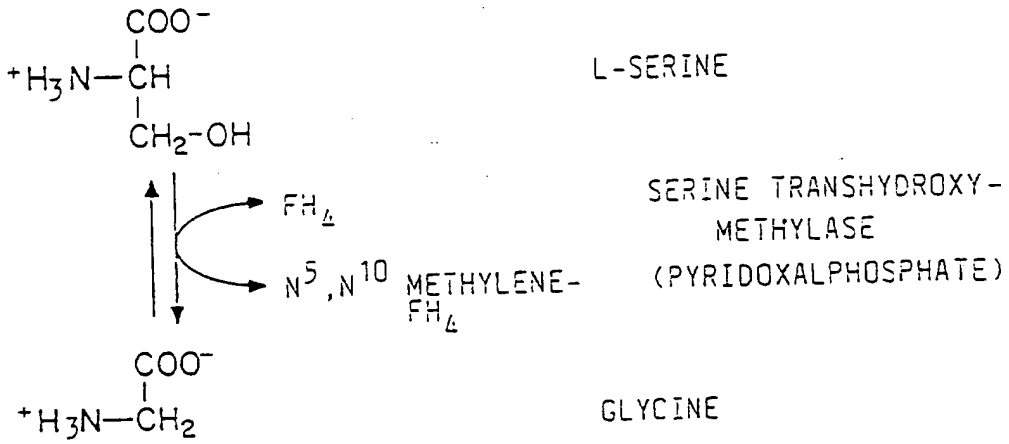

Fig. 3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+H3N—CH
CH2-OH
L-SERINE
SERINE TRANSHYDROXY-
METHYLASE
(PYRIDOXALPHOSPHATE)
FH4
N5,N10 METHYLENE-
FH4
COO⁻
+H3N—CH2
GLYCINE